amended to delete the objectionable phrase, and now more clearly describes the claimed invention wherein a reduced surface tension of the second liquid contributes to the cell disruption method.

C. Claim 8 was alleged to be vague and indefinite for essentially the same reasons as Claim 5 above. An amendment similar to that for Claim 5 is submitted herein for Claim 8.

D. Claims 9-13 were alleged to be vague and indefinite because of the lack of distinction between "a first liquid" and "a second liquid". Claims 8-11 have been amended to clearly distinguish a first liquid and a second liquid.

II. Obviousness (35 U.S.C. §103(a))

Claims 1 and 3-13 were rejected under 35 U.S.C. §103(a) as being unpatentable over previously cited Buck et al., in view of newly cited Robson et al. and newly cited Robbins et al. Buck was alleged to teach all aspects of the claimed invention. Robson and Robbins were alleged to also show the use of alkaline pH liquids and ultrasonic energy to lyse cells. It is then further asserted at page 4 of Paper No. 9 that:

> Applicant's arguments filed June 10, 1999, have been fully considered but they are not persuasive. The argument that Buck et al. neither teaches or suggests the "placing into said liquid a vessel comprising cells in a second liquid "**at an alkaline pH**" set forth in claim 1 is not persuasive since Buck et al. teaches PCR buffer (10mM Tris-HCl, pH 8.3) is added to the cells in a tube before sonification. The first liquid of Buck et al. is "a dish of water" next to the sonicator probe of which the tubes containing the cells and second liquid adjusted to an alkaline pH with PCR buffer are contained therein. Thus, this teaching by Buck et al. suggests a vessel comprising cells in a second liquid at an alkaline pH.

First, it is respectfully submitted that the allegations regarding the teaching of Buck are inaccurate. Specifically, the sonication treatment of Buck is described in the paragraph bridging pages 1331 and 1332, and a careful reading of this disclosure clearly shows that the suspensions

referenced are those described in the paragraph entitled "Type Culture of *M. tuberculosis*" in the first column on page 1331. It is these 10-fold dilution suspensions which are treated by the four methods of cell disruption: (i) treatment with proteinase K and nonionic detergents, (ii) boiling with nonionic detergents, (iii) freezing and thawing, and (iv) sonication.

Each of these four methods starts with the same 10-fold dilution suspensions. However, PCR buffer is used in only the first three methods. In the sonication method, the 10-fold dilution suspensions are centrifuged as described in method (iii) (*i.e.* 16,000 xg for 5 min.), then washed twice in distilled water and resuspended in the residual water. There is no mention of, or reference to, a PCR buffer in this method (iv).

In contrast, in each of methods (i), (ii) and (iii) of Buck, the PCR buffer is used in the specific technique being compared to the sonication of method (iv). Specifically:

(i) In method (i), the PCR buffer contains the proteinase K and nonionic detergents.

(ii) In method (ii), the PCR buffer contains the nonionic detergent and is the medium in which the mycobacterial cell suspension is boiled.

(iii) In method (iii), the PCR buffer also contains nonionic detergent and is the medium in which the mycobacterial cell suspension is subjected to freezing and thawing.

As each of the above methods is being compared by Buck to method (iv), and in each of the above methods the PCR buffer is integral to the method, method (iv) can not be reasonably read to include the PCR buffer of any of the first three methods.

Furthermore, beyond the lack of disclosure of alkalization with sonication, Buck essentially teaches away from alkalinization of the liquid in which cells are located by finding that "the sonication procedure produced the most promising results" (page 1332, first paragraph of the second column). This most promising sonication method is conducted with a non-alkalinized liquid, in distinct contrast to the other three comparative methods that utilized alkalinized liquids.

Similarly, Robson does not provide any suggestion to combine an alkalinized liquid with the "most promising" sonication method of Buck. Specifically, in the cited passages of Robson: (1) alkaline buffers are used with heat to lyse cells (column 6, lines 25-40), and (2) sonication with beads is conducted in non-alkalinized water (column 8, lines 53-65). Neither of these passages suggests to one of ordinary skill in the art, to combine an alkalinized liquid with

sonication as claimed in the present application, particularly in view of the teaching of Buck, that sonication in a non-alkalinized liquid is "most promising".

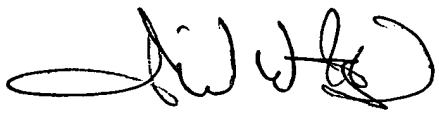
Given this teaching of Buck, one of ordinary skill in the art would not be motivated to combine such teaching with the teaching of Robson. Such a skilled artisan also would not have expected to achieve the enhanced results of alkalinized liquid with sonication reported by the Applicants in Example 2 of the present application (pages 12-15).

Moreover, the disclosure of Robbins would also not motivate the skilled artisan to derive the invention claimed in the present application. Robbins teaches that alkalization occurs after cell rupture, not as part of the cell disruption method. Specifically, it is the cell homogenate which has its pH adjusted to between 8 and 11 (column 3, lines 45-47). This alkalinization is conducted because it "extracts the nuclease, proteins and other alkali soluble materials" (column 3, lines 48-49). These nucleases, proteins and other alkali soluble materials are present for extraction because the yeast cells of Robbins have already been ruptured in a non-alkalinized liquid. Robbins' only disclosure of alkalinity prior to cell rupture is reference to incorporation of dilute alkali in a water wash of the yeast cell biomass (column 3, lines 18-20). However, such a wash will not remain as the liquid in which subsequent disruption method is conducted.

III. Conclusion

In view of the claim amendments and remarks above, the present application is believed to be in condition for allowance.

Respectfully submitted,



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